

AMENDMENTS TO THE CLAIMS

Claim 1 (currently amended): A method of vaccinating a vertebrate species, comprising administering to a vertebrate species a vaccine comprising a composition prepared by:

- (A) forming a water-in-oil emulsion comprising
 - (a) water,
 - (b) an alginate,
 - (c) an oil,
 - (d) an antigen, and
 - (e) a surfactant composition comprising at least one of
 - (i) a cellulose ether and at least one nonionic surfactant and
 - (ii) a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer surfactant and at least one nonionic surfactant;
- (B) crosslinking the alginate in the emulsion of step (A) with at least two different cations selected from the group consisting of aluminum, barium, calcium, manganese, strontium, and zinc, to form antigen-containing crosslinked alginate microparticles; and
- (C) harvesting the microparticles of step (B).

Claim 2 (original): The method of claim 1 wherein the microparticles are less than about 10 μm in diameter.

Claim 3 (original): The method of claim 1 wherein the cellulose ether is selected from the group consisting of ethylcellulose, methylcellulose, hydroxymethylcellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, and mixtures thereof.

Claim 4 (previously presented): The method of claim 1 wherein at least one of said nonionic surfactants of steps (A)(e)(i) and (ii) is selected from the group consisting of polyoxyethylene surfactants, anhydrosorbitol ester surfactants, ethoxylated anhydrosorbitol ester surfactants, and mixtures thereof.

Claim 5 (previously presented): The method of claim 4 wherein said polyoxyethylene surfactant is an alcohol ethoxylate.

Claim 6 (withdrawn): The method of claim 5 wherein said alcohol ethoxylate is polyoxyethylene (2) oyl ether.

Claim 7 (withdrawn): The method of claim 4 wherein said nonionic surfactant is an anhydrosorbitol ester.

Claim 8 (withdrawn) The method of claim 7 wherein said anhydrosorbitol ester is sorbitan trioleate.

Claim 9 (withdrawn) The method of claim 4 wherein said nonionic surfactant is an ethoxylated anhydrosorbitol ester.

Claim 10: (withdrawn) The method of claim 9 wherein said ethoxylated anhydrosorbitol ester is polyoxyethylene sorbitan trioleate.

Claim 11 (previously presented): The method of claim 1 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has from about 15 to about 70 propylene oxide residues.

Claim 12 (original): The method of claim 11 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has about 30 propylene oxide residues.

Claim 13 (original): The method of claim 11 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has the formula $(EO)_3(PO)_{30}(EO)_3$.

Claim 14 (original): The method of claim 1 wherein the antigen is selected from the group consisting of live virus, live bacteria, killed virus, killed bacteria, nucleic acids, subunit antigens of infectious agents, and mixtures thereof.

Claim 15 (original): The method of claim 1 wherein the cations comprise calcium and zinc.

Claim 16 (original): The method of claim 1 wherein said emulsion comprises sources of said cations selected from the group consisting of $AlSO_4$, $BaCl_2$, $CaCl_2$, $MnCl_2$, $ZnCl_2$, calcium acetate, zinc acetate, strontium nitrate, and mixtures thereof.

Claim 17 (original): The method of claim 16 wherein the sources of cations comprise $CaCl_2$ and $ZnCl_2$.

Claim 18 (original): The method of claim 16 wherein the sources of cations comprise calcium acetate and zinc acetate.

Claim 19 (original): The method of claim 1 wherein the emulsion of step (A) further comprises poly(propylene glycol).

Claim 20 (previously presented): The method of claim 1 wherein the emulsion of step (A) comprises said cellulose ether and at least two nonionic surfactants.

Claim 21 (previously presented): The method of claim 1 further comprising, prior to step (A), the step of adding at least one nonionic surfactant to the oil.

Claim 22 (original): The method of claim 1 comprising the step of coating the microparticles harvested in step (C) with a polymer.

Claim 23 (original): The method of claim 22 wherein the polymer is a poly-cation.

Claim 24 (original): The method of claim 23 wherein the poly-cation is selected from the group consisting of poly-L-lysine, polyhistidine, polyarginine, polyethyleneimine, and mixtures thereof.

Claim 25 (original): The method of claim 24 wherein the poly-cation is poly-L-lysine.

Claim 26 (original): The method of claim 1 comprising the step of coating the microparticles harvested in step (C) with a mucosal adjuvant/adhesant.

Claim 27 (original): The method of claim 26 wherein the mucosal adjuvant/adhesant is selected from the group consisting of lectins, cholera toxin, B subunit toxin of cholera toxin, recombinant derived subunits of B subunit toxin of cholera toxin, pertussis toxin, heat labile toxin of *E. coli*, exotoxin A of *P. aeruginosa*, and mixtures thereof.

Claim 28 (previously presented): The method of claim 1 wherein step (B) comprises adding the cations to the emulsion of step (A) in dropwise fashion, while stirring the emulsion at a speed of at least about 1,000 RPM.

Claim 29 (currently amended): The method of claim 1 wherein at least one of said nonionic ~~surfactants~~ surfactant of steps (A)(e)(i) and (ii) and said poly(ethylene oxide)-

poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has a hydrophile/lipophile balance from about 1 to about 15.

Claim 30 (previously presented): The method of claim 1 wherein said surfactant composition has a hydrophile/lipophile balance of about 8.3.

Claim 31 (withdrawn): The method of claim 1 wherein the emulsion comprises said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer and said nonionic surfactant is an ethoxylated anhydrosorbitol ester surfactant.

Claim 32 (withdrawn): The method of claim 31 wherein said ethoxylated anhydrosorbitol ester surfactant comprises polyoxyethylene sorbitan trioleate.

Claim 33 (withdrawn): The method of claim 32 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has the formula $(EO)_3(PO)_{30}(EO)_3$.

Claim 34 (withdrawn): The method of claim 33 wherein the emulsion further comprises said cellulose ether.

Claims 35-42: (cancelled).

Claim 43 (previously presented): The method of claim 1, wherein said administering is performed orally.

Claim 44 (previously presented): A method of vaccinating a vertebrate species, comprising administering to a vertebrate species a composition comprising a multi-cation cross-linked alginate comprising an antigen, a nonionic surfactant, and one or both of a cellulose ether and a poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer surfactant,

the cations of said multi-cation crosslinked alginate selected from the group consisting of aluminum, barium, calcium, manganese, strontium, and zinc.

Claim 45 (previously presented): The method of claim 44 wherein the microparticles are less than about 10 μm in diameter.

Claim 46 (previously presented): The method of claim 44 wherein the cellulose ether is selected from the group consisting of ethylcellulose, methylcellulose, hydroxymethylcellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, and mixtures thereof.

Claim 47 (currently amended): The method of claim 44 wherein ~~at least one~~ of said nonionic surfactants of steps (A)(c)(i) and (ii) surfactant is selected from the group consisting of polyoxyethylene surfactants, anhydrosorbitol ester surfactants, ethoxylated anhydrosorbitol ester surfactants, and mixtures thereof.

Claim 48 (previously presented): The method of claim 47 wherein said polyoxyethylene surfactant is an alcohol ethoxylate.

Claim 49 (previously presented): The method of claim 48 wherein said alcohol ethoxylate is polyoxyethylene (2) oyl ether.

Claim 50 (withdrawn): The method of claim 47 wherein said nonionic surfactant is an anhydrosorbitol ester.

Claim 51 (withdrawn) The method of claim 50 wherein said anhydrosorbitol ester is sorbitan trioleate.

Claim 52 (withdrawn) The method of claim 47 wherein said nonionic surfactant is an ethoxylated anhydrosorbitol ester.

Claim 53: (withdrawn) The method of claim 52 wherein said ethoxylated anhydrosorbitol ester is polyoxyethylene sorbitan trioleate.

Claim 54 (previously presented): The method of claim 44 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has from about 15 to about 70 propylene oxide residues.

Claim 55 (previously presented): The method of claim 54 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has about 30 propylene oxide residues.

Claim 56 (previously presented): The method of claim 54 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has the formula $(EO)_3(PO)_{30}(EO)_3$.

Claim 57 (previously presented): The method of claim 44 wherein the antigen is selected from the group consisting of live virus, live bacteria, killed virus, killed bacteria, nucleic acids, subunit antigens of infectious agents, and mixtures thereof.

Claim 58 (previously presented): The method of claim 44 wherein the cations comprise calcium and zinc.

Claim 59 (previously presented): The method of claim 44 wherein said emulsion comprises sources of said cations selected from the group consisting of AlSO_4 , BaCl_2 , CaCl_2 , MnCl_2 , ZnCl_2 , calcium acetate, zinc acetate, strontium nitrate, and mixtures thereof.

Claim 60 (previously presented): The method of claim 59 wherein the sources of cations comprise CaCl_2 and ZnCl_2 .

Claim 61 (previously presented): The method of claim 59 wherein the sources of cations comprise calcium acetate and zinc acetate.

Claim 62 (currently amended): The method of claim 44 wherein the ~~emulsion of step (A)~~ alginate further comprises poly(propylene glycol).

Claim 63 (currently amended): The method of claim 44 wherein the ~~emulsion of step (A)~~ alginate comprises said cellulose ether and at least two nonionic surfactants.

Claim 64 (canceled).

Claim 65 (currently amended): The method of claim 44 comprising the step of coating the ~~microparticles harvested in step (C)~~ alginate with a polymer prior to said administering.

Claim 66 (previously presented): The method of claim 65 wherein the polymer is a poly-cation.

Claim 67 (previously presented): The method of claim 66 wherein the poly-cation is selected from the group consisting of poly-L-lysine, polyhistidine, polyarginine, polyethyleneimine, and mixtures thereof.

Claim 68 (previously presented): The method of claim 67 wherein the poly-cation is poly-L-lysine.

Claim 69 (currently amended): The method of claim 44 comprising the step of coating the ~~microparticles harvested in step (C)~~ alginate with a mucosal adjuvant/adhesant prior to said administering.

Claim 70 (previously presented): The method of claim 69 wherein the mucosal adjuvant/adhesant is selected from the group consisting of lectins, cholera toxin, B subunit toxin of cholera toxin, recombinant derived subunits of B subunit toxin of cholera

toxin, pertussis toxin, heat labile toxin of *E. coli*, exotoxin A of *P. aeruginosa*, and mixtures thereof.

Claim 71 (cancelled).

Claim 72 (currently amended): The method of claim 44 wherein at least one of said nonionic surfactants of steps (A)(e)(i) and (ii) ~~surfactant~~ and said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has a hydrophile/lipophile balance from about 1 to about 15.

Claim 73 (currently amended): The method of claim 44 wherein said surfactant composition has a hydrophile/lipophile balance of about 8.3.

Claim 74 (withdrawn): The method of claim 44 wherein the ~~emulsion~~ alginate comprises said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer and said nonionic surfactant is an ethoxylated anhydrosorbitol ester surfactant.

Claim 75 (withdrawn): The method of claim 74 wherein said ethoxylated anhydrosorbitol ester surfactant comprises polyoxyethylene sorbitan trioleate.

Claim 76 (previously presented): The method of claim 75 wherein said poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer has the formula (EO)₃(PO)₃₀(EO)₃.

Claim 77 (currently amended): The method of claim 76 wherein the ~~emulsion~~ alginate further comprises said cellulose ether.

Claim 78 (previously presented): The method of claim 44, wherein said administering is performed orally.